Prognostic Impact of Platelet-Derived Growth Factors in Non-small Cell Lung Cancer Tumor and Stromal Cells

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Background: In tumor angiogenesis there is a complex interplay between endothelial, stromal, and tumor cells (neoplastic epithelial cells). Platelet-derived growth factors (PDGFs) and receptors (PDGFRs) are pivotal in this interaction, and important targets in novel antiangiogenic therapies. This study investigates the prognostic impact of these molecular markers in tumor cells and tumor stroma of resected non-small cell lung cancer (NSCLC) tumors.

Methods: Tumor tissue samples from 335 resected patients with stage I to IIIA NSCLC were obtained and tissue microarrays were constructed from duplicate cores of tumor cells and tumor-related stroma from each specimen. Immunohistochemistry was used to evaluate the expression of the molecular markers PDGF-A, -B, -C, and -D and PDGFR-α and -β.

Results: In univariate analyses, high tumor cell expression of PDGF-B ($p = 0.001$), PDGF-C ($p = 0.01$), and PDGFR-α ($p = 0.026$) were negative prognostic indicators for disease-specific survival. In tumor stroma, high expression of PDGF-A ($p = 0.009$), PDGF-B ($p = 0.04$), PDGF-D ($p = 0.019$), and PDGFR-α ($p = 0.019$) correlated with good prognosis. In multivariate analyses, high tumor cell PDGF-B ($p = 0.001$) and PDGFR-α ($p = 0.047$) expression were independent negative prognostic factors for disease-specific survival, whereas in stromal cells high PDGF-A ($p = 0.001$) expression had an independent positive survival impact.

Conclusion: Our results indicate PDGF-B and PDGFR-α inhibition as an interesting approach in NSCLC treatment, but also demonstrates the importance of understanding the cellular crosstalk between endothelial, stromal, and tumor cells when targeting PDGF markers.

Key Words: NSCLC, Angiogenesis, PDGFs, PDGFRs, Stroma.

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Lung cancer is the leading cause of cancer-related mortality in both men and women.[1] Although chemotherapy recently has shown promising results in the adjuvant clinical setting and there has been some progress in the treatment of locally advanced and advanced disease, treatment outcomes for non-small cell lung cancer (NSCLC) patients are in general disappointing.[2–5] Thus, clinical research on new treatment strategies is warranted. In the pipeline for new NSCLC therapies, several agents involve directly or indirectly platelet-derived growth factors (PDGFs) and its receptors (PDGFRs), e.g., sorafenib, sunitunib, imatinib, and bevacizumab.[6,7]

The PDGF family consists of five isoforms of A-, B-, C-, and -D polypeptide chains that is homodimers PDGF-AA, -BB, -CC, -DD, and a hetereodimer PDGF-AB.[8,9] The PDGF isoforms exert their cellular effects by binding to structurally similar α- and β-tyrosine kinase PDGF receptors. PDGF-AA, -AB, -BB, and CC dimers bind to the α-receptor with high affinity, whereas PDGF-BB and -DD dimers bind preferentially to the β-receptor.[10,11]

PDGF ligands and receptors are of major importance in angiogenesis.[10–12] PDGF-B and PDGFR-β is required for recruitment of pericytes, which are periendothelial smooth muscle-like cells, which modulate endothelial cell function, and in maturation of the microvasculature.[13] Recent studies have emphasized the significance of tumor-derived PDGF-A (and potentially PDGF-C) and PDGFR-α signaling in recruitment of the angiogenic stroma to produce vascular endothelial growth factor-A (VEGF-A), and other angiogenic factors.[14]

To our knowledge, only two previous studies have explored the prognostic relevance of PDGFs/PDGFRs expression and survival in NSCLC patients.[15,16] Kawai et al.[15] found tumor cell PDGF-B expression, by immunohistochemistry (IHC), to predict a negative outcome in 92 resected specimens whereas Shikada et al.[16] observed that PDGF-A had a negative prognostic impact in univariate analysis. In the latter study, they also found PDGF-A to be an autocrine (signaling affects cells of the same type as secreted), and probably paracrine stimulator (signaling in which the target cell is a different cell type close to the signal releasing cell), yielding an essential contribution to the expression of vascular endothelial growth factor (VEGF).

As new agents, involving PDGF/PDGFR inhibition, are being clinically evaluated for NSCLC treatment, further knowledge about these ligands and receptors and the complex interplay between endothelial, stromal, and tumor cells is

warranted. We have previously reported on the importance of VEGFs and their receptors in both tumor cells and stroma.[17] This is the first report on the prognostic significance of PDGF-A, -B, -C, and -D and PDGFR-α and -β expression in both tumor cells and in stroma of resected NSCLC patients.

PATIENTS AND METHODS

Patients and Clinical Samples

Primary tumor tissues from anonymized patients diagnosed with NSCLC pathologic stage I to IIIA at the University Hospital of Northern Norway and Nordland Central Hospital from 1990 through 2004 were used in this retrospective study. In total, 371 patients were registered from the hospital database. Of these, 36 patients were excluded from the study because of: (i) Radiotherapy (RT) or chemotherapy before surgery ($n = 10$); (ii) Other malignancy within 5 years before NSCLC diagnosis ($n = 13$); (iii) Inadequate paraffin-embedded fixed tissue blocks ($n = 13$). Adjuvant chemotherapy was not introduced in Norway during this period (1990–2004). Thus, 335 patients with complete medical records and adequate paraffin-embedded tissue blocks were eligible.

This report includes follow-up data as of September 30, 2005. The median follow-up was 96 (range, 10–179) months. Complete demographic and clinical data were collected retrospectively. Formalin-fixed and paraffin-embedded tumor specimens were obtained from the archives of the Departments of Pathology at University Hospital of Northern Norway and Nordland Central Hospital. The tumors were staged according to the International Union Against Cancer's tumor, node, metastasis classification and histologically subtyped and graded according to the World Health Organization guidelines.[18] The National Data Inspection Board and The Regional Committee for Research Ethics approved the study.

Microarray Construction

All lung cancer cases were histologically reviewed by two pathologists (S.A.-S. and K. A.-S.) and the most representative areas of tumor cells (neoplastic epithelial cells) and tumor stroma were carefully selected and marked on the hematoxylin and eosin slide, and sampled for the tissue microarray blocks (TMAs). The TMAs were assembled using a tissue-arraying instrument (Beecher Instruments, Silver Springs, MD). The detailed methodology has been previously reported.[17] Briefly, we used a 0.6 mm diameter stylet, and the study specimens were routinely sampled with two replicate core samples (different areas) of neoplastic tissue and two of tumor stroma. Both normal lung tissue localized distant from the primary tumor, and one slide with normal lung tissue samples from 20 patients without a cancer diagnosis, were used as negative controls.

To include all core samples, eight tissue array blocks were constructed. Multiple 5-μm sections were cut with a Micron microtome (HM355S) and stained by specific antibodies for IHC analysis.

Immunohistochemistry

The applied antibodies were subjected to in-house validation by the manufacturer for IHC analysis on paraffin-embedded material. The antibodies used in the study were as follows: PDGF-AA (goat polyclonal; AB-221-NA; R and D Systems; 1:200), PDGF-AB/BB (rabbit polyclonal; RB-9257; Neomarkers; 1:15), PDGF-CC (goat polyclonal; GT15151; Neuromics; 1:80), PDGF-DD (goat polyclonal; AF1159; R and D Systems; 1:400), PDGFR-α (rabbit polyclonal; RB-9027; Neomarkers; 1:75), and PDGFR-β (rabbit polyclonal; RB-9032; Neomarkers; 1:25).

Sections were deparaffinised with xylene and rehydrated with ethanol. Regarding ligands (PDGF-A, -B, -C, and -D), antigen retrieval was performed by placing the specimen in 0.01 M citrate buffer at pH 6.0 and exposed to repeated ($\times$ 2) microwave heating of 10 minutes at 450W. PDGF-A, -B, and -C were stained using peroxydase/diamino benzidine (Dako EnVision + System-Horseadish peroxidase/diamino benzidine). The primary antibodies were incubated for 30 minutes in room temperature. PDGF-D was visualized by adding a secondary antibody conjugated with Biotin, followed by an avidin/biotin/peroxydase complex (Vectastein avidin-biotin-peroxidase complex Elite kit from Vector Laboratories). The primary antibody was incubated over night at 4°C. Finally, all slides were counterstained with hematoxylin to visualize the nuclei.

The receptors (PDGFR-α and -β) were stained using Ventana BenchMark XT (Ventana Medical Systems Inc.), procedure iView Diamino benzidine. Antigen retrieval was done in Tris/ethylenediaminetetraacetic acid buffer at pH 8.4 for 30 minutes (PDGFR-α) or 60 minutes (PDGFR-β) at 37°C. The primary antibodies were incubated for 30 minutes in room temperature.

For each antibody, included negative staining controls, all TMA stainings were done in a single experiment.

Scoring of Immunohistochemistry

By light microscopy, representative and viable tissue sections were scored semiquantitatively for cytoplasmic staining. The dominant staining intensity in both tumor cells and stromal cells was scored as: 0 = negative; 1 = weak; 2 = intermediate; 3 = strong (Figure 1). The cell density of the stroma was scored as: 1 = low density; 2 = intermediate density; 3 = high density (Figure 1). All samples were anonymized and independently scored by two pathologists (S.A.-S. and K.A.-S.). In case of disagreement, the slides were reexamined and a consensus was reached by the observers. In most tumor cores and in some stromal cores there is a mixture of stromal cells and tumor cells. Nevertheless, by morphologic criteria we have only scored staining intensity of tumor cells in tumor cores and intensity and density of stromal cells in stromal cores. When assessing a variable for a given core, the observers were blinded to the scores of the other variables and to outcome. The interobserver scoring agreement has been previously found valid in the same TMA-blocks for one ligand and one receptor with similar cytoplasmic staining.[17] After categorizing into high and low expression group, the percentage discordance among the pathologists was tumor cell ligand 8%, stromal ligand 8%, tumor cell receptor 2%, and stromal receptor 4%. Mean score for duplicate cores from each individual was calculated separately in tumor cells and stroma. High expression in tumor

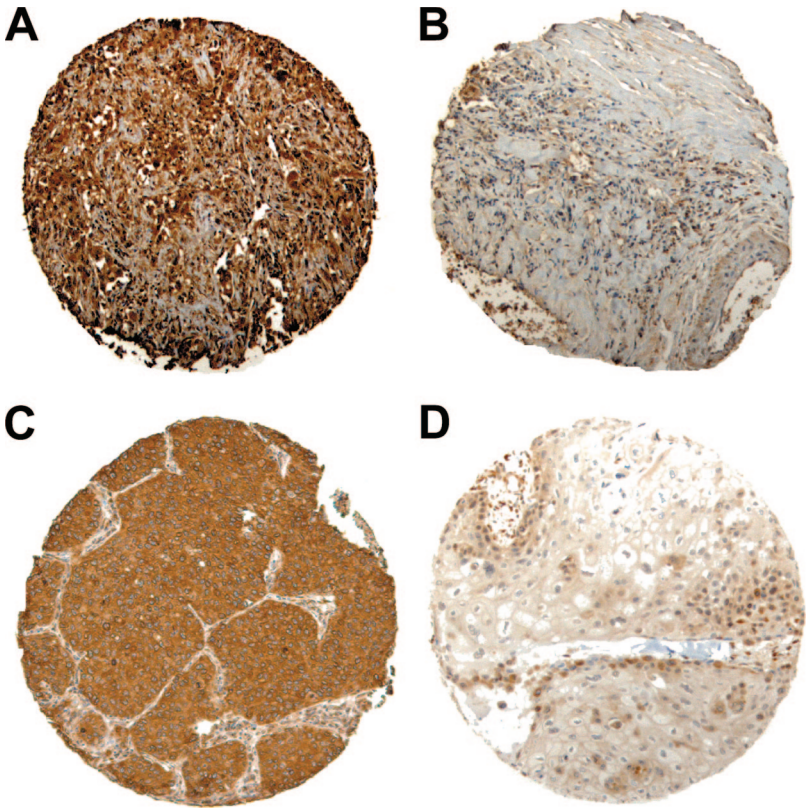

FIGURE 1. Immunohistochemical analysis of TMA of NSCLC representing different score for tumor cell PDGFR-α and PDGF-A; *A,* Stromal PDGF-A high score (density 3, intensity 3); *B,* Stromal PDGF-A low score (density 1, intensity 0); *C,* Tumor cell PDGFR-α score 3; *D,* Tumor cell PDGFR-α score 1. TMA, tissue microarray block; NSCLC, non-small cell lung cancer; PDGF, platelet-derived growth factor.

cells was defined as score ≥1.5 (PDGF-C), ≥2 (PDGF-A, PDGFR-α, and PDGFR-β), or = 3 (PDGF-B and PDGF-D). Stromal expression was calculated by summarizing density score (1–3) and intensity score (0–3) before categorizing into low and high expression. High expression in stroma was defined as score ≥2.5 (PDGFR-β), ≥4 (PDGF-B), ≥4.5 (PDGF-A, PDGF-C, and PDGFR-α), or ≥5.5 (PDGF-D).

Statistical Methods

All statistical analyses were done using the statistical package SPSS (Chicago, IL), version 14. The χ^2 test and Fisher exact test were used to examine the association between molecular marker expression and various clinicopathological parameters. Univariate analysis was done by using the Kaplan-Meier method. Statistical significance between survival curves was assessed by the log-rank test. Disease-specific survival (DSS) was determined from the date of surgery to the time of lung cancer death. To assess the independent value of different pretreatment variables on survival, in the presence of other variables, multivariate analysis was carried out using the Cox proportional hazards model. Only variables of significant value from the univariate analysis were entered into the Cox regression analysis. Probability for stepwise entry and removal was set at 0.05 and 0.10, respectively.

RESULTS

Clinocopathologic Variables

Demographic, clinical, and histopathologic variables are shown in Table 1. The median age was 67 (range, 28–85) years and the majority of patients were male (76%). The NSCLC tumors comprised 191 squamous cell carcinomas (SCCs), 95 adenocarcinomas, 31 large-cell carcinomas (LCCs), and 18 bronchioalveolar carcinomas (BACs). Because of nodal metastasis or nonradical surgical margins, 59 (18%) patients received postoperative RT.

Expression of Platelet-Derived Growth Factors/ Platelet-Derived Growth Factor Receptors and Their Correlations

PDGFs/PDGFRs were expressed in the cytoplasm of tumor cells. On the basis of only morphologic criteria without any double IHC, we estimated more than 50% of normal pneumocytes in control cores to be stained by all antibodies, except PDGFR-α where less than 50% were stained. In tumor stroma and in control cores, more than 50% of inflammatory cells (macrophages, lymphocytes, granulocytes, and plasma cells) were stained, except for PDGFR-β where less than 50% were stained. Nearly all fibroblast-like cells were stained, only PDGFR- α stained less than 50% of these cells. In control cores, most vascular endothelium was stained for PDGF-A, -B, and -D and PDGFR-β, less than 50% for PDGF-C and none for PDGFR-α. The majority of vascular smooth muscle cells were stained, except for PDGFR-α and PDGFR-β where less than 50% were stained.

No correlation was observed between tumor or stromal cell PDGFs/PDGFRs expression versus age, performance status, weight loss, tumor differentiation, or vascular infiltration. Among the ligands, tumor cell PDGF-A was less frequently expressed in LCC (high expression; LCC 23%, BAC

TABLE 1. Prognostic Clinicopathologic Variables as Predictors for Disease-Specific Survival in 335 NSCLC Patients (Univariate Analysis; Log-Rank Test)

Characteristic	Patients (*n*)	Patients (%)	Median Survival (mo)	5-Year Survival (%)	*p*
Age					
≤65 yr	156	47	104	57	0.62
>65 yr	179	53	NR	58	
Sex					
Female	82	25	127	65	0.19
Male	253	75	84	55	
Smoking					
Never	15	5	19	43	0.13
Current	215	64	NR	60	
Former	105	31	84	54	
Performance status					
ECOG 0	197	59	NR	62	0.04
ECOG 1	120	36	61	52	
ECOG 2	18	5	36	40	
Weight loss					
<10%	303	90	127	57	0.92
>10%	32	10	NR	57	
Histology					
SCC	191	57	NR	65	0.30
Adenocarcinoma	95	28	52	44	
BAC	18	5	NR	67	
LCC	31	9	84	54	
Differentiation					
Poor	138	41	48	48	0.001
Moderate	144	43	NR	64	
Well	53	16	NR	65	
Surgical procedure					
Lobectomy + Wedge[a]	243	73	NR	61	0.0009
Pneumonectomy	92	27	35	46	
Stage					
I	212	63	NR	68	<0.0001
II	91	27	41	46	
IIIa	32	10	18	22	
Tumor status					
1	90	27	NR	75	0.002
2	218	65	84	52	
3	27	8	42	43	
Nodal status					
0	232	69	NR	66	<0.0001
1	76	23	37	43	
2	27	8	18	20	
Surgical margins					
Free	307	92	127	58	0.34
Not free	28	8	64	51	
Vascular infiltration					
No	284	85	NR	61	0.0005
Yes	51	15	25	35	
Postoperative radiotherapy					
No	276	82	NR	61	0.002
Yes	59	18	41	42	

[a] Wedge, $n = 10$.

NSCLC, non-small cell lung cancer; ECOG, Eastern Cooperative Oncology Group; SCC, squamous cell carcinoma; BAC, bronchioalveolar carcinoma; LCC, large-cell carcinoma; NR, not reached.

45%, SCC 56%, adenocarcinomas 56%, $p = 0.002$) and less often in pathologic stage I (high expression; p-stage I 45%, p-stage II 63%, p-stage III 67%, $p = 0.004$) Stromal PDGF-C was more often expressed in women (high expression; women 44%, men 30%, $p = 0.029$) whereas stromal PDGF-D expression was observed more frequently in LCC (high expression; LCC 23%, BAC 5%, SCC 6%, adenocarcinomas 13%, $p = 0.002$) and in p-stage I (high expression; p-stage I 13%, p-stage II 4%, p-stage III 3%, $p = 0.032$). Among receptors, tumor cell PDGFR-α expression was more often seen in women (high expression; women 71%, men 57%, $p = 0.024$) and less often in SCC (high expression; LCC 77%, BAC 68%, SCC 51%, adenocarcinomas 73%, $p < 0.001$). Stromal PDGFR-α was more often expressed in SCC (high expression; LCC 38%, BAC 35%, SCC 48%, adenocarcinomas 28%, $p = 0.046$) and stromal PDGFR-β more often in women (high expression; women 33%, men 17%, $p = 0.003$).

Univariate Analysis

As shown in Table 1, the clinical variables performance status ($p = 0.04$), differentiation ($p = 0.001$), surgical procedure ($p = 0.0009$), stage ($p < 0.0001$), histologic T-stage ($p = 0.002$), N-stage ($p < 0.0001$), vascular infiltration ($p = 0.0005$), and postoperative RT ($p = 0.002$) were all prognostic indicators for DSS. The influence on survival by tumor cell and stromal PDGF ligands and receptors are given in Table 2 and in Figure 2. In univariate analysis, tumor cell expression of PDGF-B ($p = 0.001$; Figure 2*B*), PDGF-C ($p = 0.01$) and PDGFR-α ($p = 0.026$; Figure 2*C*) and stromal expression of PDGF-A ($p = 0.009$; Figure 2*A*), PDGF-B ($p = 0.04$), PDGF-D ($p = 0.019$), and PDGFR-α ($p = 0.01$) were prognostic indicators for DSS.

Multivariate Cox Proportional Hazards Analysis

Results of the multivariate analysis are presented in Table 3. Including all significant clinicopathological and angiogenic variables from the univariate analysis, tumor cell PDGF-B ($p = 0.001$) and PDGFR-α ($p = 0.047$) expression, stromal cell PDGF-A expression ($p = 0.001$), performance status ($p = 0.013$), histologic T-stage ($p = 0.002$), N-stage ($p < 0.001$) and vascular infiltration ($p = 0.003$) appeared as independent prognostic factors. High tumor cell PDGF-C expression tended towards an independent negative impact on survival, but did not reach statistical significance ($p = 0.13$, OR 1.90, confidence interval 95% 0.82–4.38).

Correlations Between Platelet-Derived Growth Factors/Platelet-Derived Growth Factor Receptors and Vascular Endothelial Growth Factors/Vascular Endothelial Growth Factor Receptors

We have previously reported results from this cohort on VEGFs and vascular endothelial growth factor receptors (VEGFRs).[17] There are significant correlations between PDGF-A and VEGF-A expression in tumor cell ($r = 0.29$, $p < 0.001$; Table 4), between PDGF-A and VEGF-A in stroma ($r = 0.30$, $p < 0.001$; Table 5), but not between tumor cell PDGF-A and stromal VEGF-A ($r = 0.005$, $p = 0.92$). There was also a significant correlation between VEGF-A

TABLE 2. Tumor Cell and Stromal Angiogenic Markers as Predictors for Disease-Specific Survival in 335 NSCLC Patients (Univariate Analysis; Log-Rank Test)

Marker Expression	Patients (*n*)	Patients (%)	Median Survival (mo)	5-Year Survival (%)	*p*
PDGF-AA					
Tumor					
Low	158	47	NR	62	0.144
High	169	51	83	55	
Missing	8	2			
Stroma					
Low	207	62	71	52	0.009
High	125	37	NR	68	
Missing	3	1			
PDGF-AB/BB					
Tumor					
Low	282	84	NR	60	0.001
High	52	16	30	44	
Missing	1	0			
Stroma					
Low	210	63	83	53	0.04
High	125	37	NR	70	
PDGF-CC					
Tumor					
Low	41	12	NR	82	0.01
High	288	86	84	55	
Missing	6	2			
Stroma					
Low	218	65	127	56	0.29
High	113	34	NR	62	
Missing	4	1			
PDGF-DD					
Tumor					
Low	308	91	NR	60	0.20
High	22	7	42	41	
Missing	5	2			
Stroma					
Low	299	89	84	55	0.019
High	32	10	NR	86	
Missing	4	1			
PDGFR-α					
Tumor					
Low	129	39	NR	67	0.026
High	198	59	71	53	
Missing	8	2			
Stroma					
Low	192	57	71	53	0.032
High	136	41	NR	65	
Missing	7	2			
PDGFR-β					
Tumor					
Low	288	86	NR	59	0.84
High	42	12	127	51	
Missing	5	2			
Stroma					
Low	262	78	127	57	0.16
High	69	21	NR	63	
Missing	4	1			

NSCLC, non-small cell lung cancer; PDGF, platelet-derived growth factor.

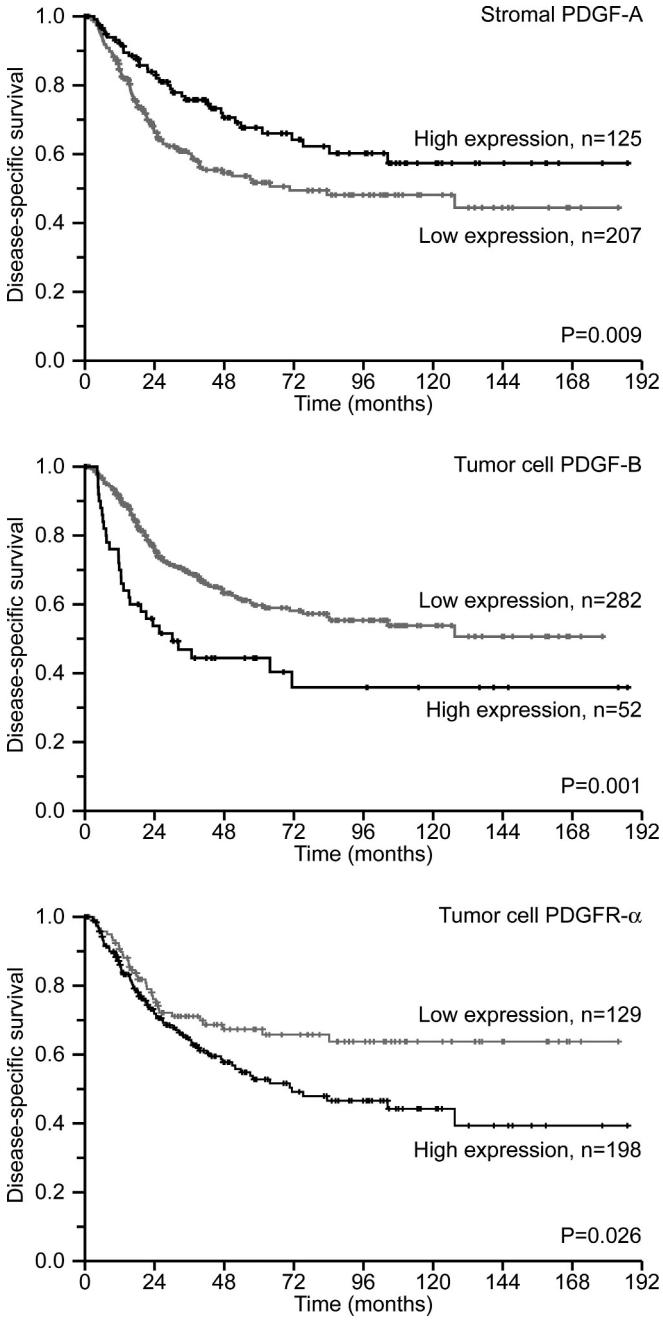

FIGURE 2. Disease-specific survival curves according to stromal PDGF-A expression, tumor cell PDGF-B expression, and tumor cell PDGFR-α expression. PDGF, platelet-derived growth factor.

and PDGF-C in stroma ($r = 0.20$, $p < 0.001$), but no correlation between VEGF-A and PDGF-C expression in tumor cells ($r = 0.08$, $p = 0.17$) or tumor cell VEGF-A and stromal PDGF-C ($r = 0.05$, $p = 0.29$).

DISCUSSION

We present a large-scale study using high-throughput TMA analyses, to examine the prognostic impact of PDGF-A,

TABLE 3. Results of Cox Regression Analysis Summarizing Significant Independent Prognostic Factors

Factor	Hazard Ratio	95% CI	*P*
Tumor status			
1	1.00		0.002[a]
2	2.04	1.23–3.38	0.005
3	3.24	1.61–6.55	0.001
Nodal status			
0	1.00		<0.001[a]
1	2.05	1.34–3.14	0.001
2	2.71	1.53–4.81	0.001
Performance status			
ECOG 0	1.00		0.013[a]
ECOG 1	1.81	1.22–2.70	0.003
ECOG 2	1.34	0.56–3.20	0.52
Vascular infiltration			
No	1.00		
Yes	2.07	1.27–3.36	0.003
PDGF-AB/BB Tumor			
Low	1.00		
High	2.12	1.35–3.31	0.001
PDGFR-α Tumor			
Low	1.00		
High	1.52	1.01–2.30	0.047
PDGF-AA Stroma			
Low	2.00	1.33–3.01	0.001
High	1.00		

[a] Overall significance as a prognostic factor.

CI, Confidence interval; ECOG, Eastern Cooperative Oncology Group; PDGF, platelet-derived growth factor.

TABLE 4. Crosstabs Showing the Correlation Between Tumor Cell VEGF-A and Tumor Cell PDGF-A

	Tumor Cell VEGF-A		
	Low Expression	High Expression	Total
Tumor cell PDGF-A			
Low expression	113	44	157
High expression	73	96	169
Total	186	140	326

Spearman correlation, $r = 0.29$, $\kappa = 0.29$, $p < 0.001$. VEGF, vascular endothelial growth factor; PDGF, platelet-derived growth factor.

TABLE 5. Crosstabs Showing the Correlation Between Stromal VEGF-A and Stromal PDGF-A

	Stromal VEGF-A		
	Low Expression	High Expression	Total
Stromal PDGF-A			
Low expression	197	10	207
High expression	93	32	125
Total	290	42	332

Spearman correlation, $r = 0.30$, $\kappa = 0.24$, $p < 0.001$. VEGF, vascular endothelial growth factor; PDGF, platelet-derived growth factor.

-B, -C, and -D and PDGFR-α and -β in both tumor cell and stroma in an unselected population of surgically resected NSCLC patients. High tumor cell PDGF-B and PDGFR-α expression were independent negative prognostic indicators for DSS, whereas high stromal PDGF-A expression correlated with a good prognosis.

To our knowledge, this is the first prognostic evaluation of PDGFR-α expression in NSCLC patients. Previous studies in tumors as gastrointestinal stromal tumor,[19] glioma,[20] and rhabdomyosarcoma[21] have shown PDGFR-α overexpression to correspond with a poor prognosis. As shown in Figure 3, PDGF ligands and receptors may be tumorigenic through both autocrine mechanisms, which drive cancer cell proliferation and by activating stromal cells in a paracrine fashion.[10–12] In a model of human lung carcinoma, Tejada et al.[22] demonstrated tumor-driven paracrine PDGFR-α signaling to be a key determinant of stromal recruitment. We observed PDGFR-α expression in viable neoplastic cells to be an independent prognostic factor. Consistent with previous studies in gliomas and sarcomas,[23,24] our results may indicate a similar PDGFR-α autocrine signaling in NSCLC tumors, though our findings may also be explained by a paracrine stimulation of PDGFR-α in the tumor cells.

Both PDGF-A and -C are believed to promote angiogenesis by stimulating VEGF-A production in the stromal cells (Figure 3). This is in addition to the contribution of VEGF-A by tumor cells. The role of PDGF-A has also been studied by Shikada et al.,[16] using cell lines and surgical specimen of human NSCLCs. The authors demonstrated PDGF-A as an autocrine, and probably also paracrine regulator, making an essential contribution to the VEGF expression in NSCLC. Our data show that tumor cell PDGF-A correlate with tumor cell VEGF-A and stromal PDGF-A with stromal VEGF-A. This may be explained by PDGF-A as a possible autocrine stimulator of VEGF-A in both tumor cells and in stromal cells.

Consistent with a study by Kawai et al.[15] in 92 NSCLC patients, we identified tumor cell PDGF-B expression to be an independent negative prognostic factor. The underlying mechanism behind this association is still not resolved. Nevertheless, studies on sophisticated mouse genetics have demonstrated that proper PDGF receptor-dependent pericyte recruitment relies on endothelial production of PDGF-B.[25] Whether PDGF-B has a direct effect on endothelial cells remain unclear.[11] On the Basis of our results, although lacking data on PDGFR- β expression in pericytes, one might speculate whether tumor cell PDGF-B also contributes through PDGF-B/PDGFR-β activation to stimulate pericytes during blood-vessel maturation. Another explanation to PDGF-B's negative prognostic impact is its possible contribution to increased interstitial fluid pressure (IFP). Although the precise mechanism behind increased IFP remains obscure, evidence from preclinical studies indicate that PDGF-B induces active participation of connective tissue cells in controlling the IFP by altering the tension of the structures in the extracellular matrix (e.g., by stimulation of fibroblasts).[26] In NSCLC xenografts exposed to imatinib, Vlahovic et al.[27] demonstrated a significant decrease in en-

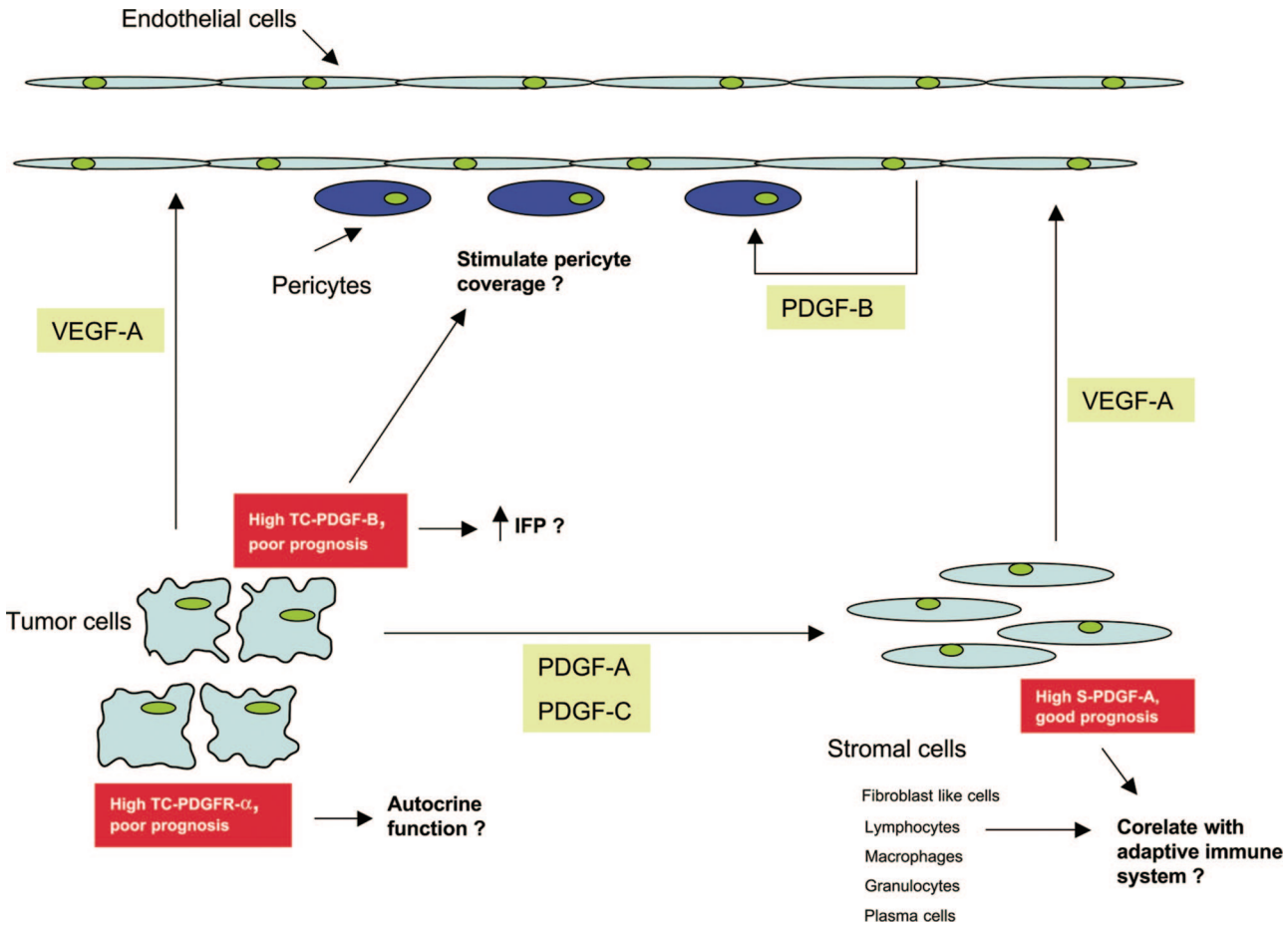

FIGURE 3. PDGFs/PDGFRs role in the complex interplay between endothelial, tumor and stromal cells in angiogenesis. Yellow boxes: Summarizing some of previous findings.[10–12] Red boxes: Our main results. PDGF, platelet-derived growth factor; VEGF, vascular endothelial growth factor; TC, tumor cell; S, stromal; IFP, interstitial fluid.

dothelial cell density and a trend towards a decreased fraction of pericyte coverage. The authors concluded that this possibly resulted from imatinib inhibition of PDGF-B/PDGFR-β through modified vasculature and decreased IFP.

The prognostic clinical significance of PDGF-C and -D expression in NSCLC patients is herewith presented for the first time. We identified tumor cell PDGF-C expression as a negative prognostic factor in the univariate analysis. Tejada et al.[22] showed that PDGF-C expression is significantly upregulated in human lung tumors indicating tumor progression through PDGFR-α signaling. Our collective data[17] also revealed high stromal PDGF-C expression to correlate with high stromal VEGF-A expression, indicating a possible PDGF-C induced stromal VEGF-A formation.

Newly formed vessels, whether they are tumor-associated or not are particularly vulnerable to VEGF-A blockade. On the other hand, mature endothelial cells, covered by extracellular matrix and pericytes, may be resistant to VEGF-A blockade or other antiangiogenic agents.[12] This was early questioned in a pioneering study by Bergers et al.,[28] demonstrating that enhanced antiangiogenic effects could be achieved by combining VEGF- and PDGF-antagonists, thereby obtaining simultaneous antiendothelial and antipericyte effects. This approach has been further developed by inclusion of drugs directed predominantly against the epithelial compartment of tumors.[29] In NSCLC patients, Bauman et al.[6] combined bevacizumab and imatinib in patients without progression from platinum doublet chemotherapy plus bevazicumab. The aim was to test if targeting PDGFR and VEGF simultaneously is a more effective antiangiogenic strategy than anti-VEGF alone. Multikinase inhibitors also target angiogenesis through this dual approach. Sorafenib is inhibiting both VEGFR-2, -3, and PDGFR-β, whereas sunitinib targets both PDGFRs and VEGFRs.[7] Evaluations of these agents in clinical phase II NSCLC studies show promising efficacy. This corroborate our previous[17] and present findings of a significantly negative role of tumor cell VEGF-A, VEGFRs, PDGFR-α, and PDGF-B.

In contrast to the study by Shikada et al.,[16] we did not observe tumor cell PDGF-A expression as a significantly negative prognostic factor for NSCLC patients. On the other hand, we present novel data showing stromal PDGF-A expression as an independent positive prognostic factor in NSCLC patients. The underlying mechanism to this somewhat surprising finding is debatable. This result also contradicts a close interplay between tumor cell and tumor-related

stroma as its receptor, the PDGFR-α, is highly expressed on the neighboring tumor cells and it is classified as a negative prognostic indicator for DSS. Though the presented stromal expression for each specific marker is the total expression of different stromal cells, including lymphocytes, macrophages, granulocytes, and fibroblast-like cells. Consequently, a possible explanation may be that the stromal PDGF-A expression is linked to one or more stromal cell types. We know that the immune system plays paradoxical roles in the defense against malignancy. In general, activation of the adaptive immune system may suppress malignant cells, whereas activation of various types of innate immune cells may promote tumor growth.[30] Though based on only morphologic criteria without any double IHC, we estimated more than 50% of our stromal lymphocytes to express PDGF-A, hence high stromal PDGF-A may to some extent reflect activation of the adaptive immune system.

In the complex interplay between endothelial, stromal and tumor cells, PDGF ligands and receptors are regarded as major players in tumor development. Beyond confirming the negative prognostic impact by tumor cell PDGF-B expression, we have demonstrated for the first time that tumor cell PDGFR-α expression is an independent negative prognostic factor in NSCLC patients. Although further investigation is needed to explain the underlying mechanisms, we have documented stromal PDGF-A expression as a positive independent prognostic factor in NSCLC. As the cellular crosstalk between endothelial, stromal and tumor cells combined is important, targeting different cellular entities by VEGF and PDGF related agents in combination with chemotherapy seems to be an appealing approach.

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